PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 December 2000 (22.12.00)	
International application No. PCT/NL00/00268	**Applicant's or agent's file reference** P49375PC00
International filing date (day/month/year) 26 April 2000 (26.04.00)	**Priority date** (day/month/year) 26 April 1999 (26.04.99)
Applicant VAN DER GREEF, Jan et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 November 2000 (21.11.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
P49375PC00		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 00/ 00268	26/04/2000	26/04/1999
Applicant		
SCREENTEC B.V.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   IDENTIFICATION OF LIGANDS FOR ORPHAN RECEPTORS USING MASS SPECTROMETRY

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the dat of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with th abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 11-12

Present claims 11-12 relate to a compound defined by reference to a desirable property, namely its ability to bind to a an (unnamed) orphan receptor.

No technical features of the compounds are present in the above-mentioned claims which would lead to this desirable property, the technical features formulated so as to permit the execution of a meaningful search. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found for only a very small proportion of the substances which could fall within the scope of these claims. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. No means are present in the above-mentioned claims by which compounds known in the prior art could be distinguished from novel substances. No definition of the subject matter for which protection is sought is therefore derivable from these claims (Article 6 PCT) or the description (Article 5 PCT). Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00268

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/566 G01N30/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NEDVED, MICHAEL L.; HABIBI-GOUDARZI, SOHRAB; GANEM, BRUCE; HENION,: "Characterization of Benzodiazepine "Combinatorial" Chemical Libraries by Online Immunoaffinity Extraction, Coupled Column HPLC-Ion Spray Mass Spectrometry-Tandem Mass Spectrometry" ANALYTICAL CHEMISTRY, vol. 68, no. 23, 1 December 1996 (1996-12-01), pages 4226-4236, XP002117165 cited in the application page 4228 -page 4231 --- -/--	1,3-9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2000	02/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hart-Davis, J

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SIEGEL, MARSHALL M.; TABEI, KEIKO; BEBERNITZ, GERALDINE A.; BAUM, ELLEN Z.: "Rapid methods for screening low molecular mass compounds non-covalently bound to proteins using size exclusion and mass spectrometry applied to inhibitors of human cytomegalovirus protease" JOURNAL OF MASS SPECTROMETRY, vol. 33, no. 3, 1998, pages 264-273, XP002117166 cited in the application the whole document	1,3-9
X	DUNAYEVSKIY, YURIY M.; LAI, JAN-JI; QUINN, CHERYL; TALLEY, FRANK; VOUROS, PAUL: "Mass spectrometric identification of ligands selected from combinatorial libraries using gel filtration" RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 11, no. 11, 1997, pages 1178-1184, XP002117167 cited in the application the whole document	1,3-9
X	BLOM, KARL F.; LARSEN, BARBARA S.; MCEWEN, CHARLES N.: "Determining Affinity-Selected Ligands and Estimating Binding Affinities by Online Size Exclusion Chromatography/Liquid Chromatography-Mass Spectrometry" JOURNAL OF COMBINATORIAL CHEMISTRY, vol. 1, no. 1, January 1999 (1999-01), pages 82-90, XP002117168 cited in the application page 82 -page 85	1,3-9
X	HUYER, GREGORY; KELLY, JOHN; MOFFAT, JASON; ZAMBONI, ROBERT; JIA, ZONGCHAO; GRESSER, MICHAEL J.; RAMACHANDRAN, CHIDAMBARAM: "Affinity selection from peptide libraries to determine substrate specificity of protein tyrosine phosphatases" ANALYTICAL BIOCHEMISTRY, vol. 258, no. 1, 1998, pages 19-30, XP002117169 cited in the application page 21	1,3-9

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00268

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	HSIEH YF, GORDON N, REGNIER F, AFEYAN N, MARTIN SA, VELLA GJ: "Multidimensional chromatography coupled with mass spectrometry for target-based screening" MOLECULAR DIVERSITY, vol. 2, no. 4, April 1997 (1997-04), pages 189-196, XP002117170 cited in the application figures 1-7	1-9
A	KAUR S, MCGUIRE L, TANG D, DOLLINGER G, HUEBNER V: "Affinity selection and mass spectrometry-based strategies to identify lead compounds in combinatorial libraries" JOURNAL OF PROTEIN CHEMISTRY, vol. 16, no. 5, July 1997 (1997-07), pages 505-511, XP002117171 cited in the application the whole document	1-9
A	WO 98 56028 A (ARQULE INC ;KYRANOS JAMES N (US); LI YU TYSR (US)) 10 December 1998 (1998-12-10) examples 1,2	1-9
A	WO 97 01755 A (PERSEPTIVE BIOSYSTEMS INC) 16 January 1997 (1997-01-16) claim 1; example 1	1-9
A	WO 97 43301 A (CIBA GEIGY AG ;GOELLER CHRISTINE (US); KELLY MICHELE ANN (US); THO) 20 November 1997 (1997-11-20) claim 1	1-9
A	WO 96 37777 A (NELSON RANDALL W ;WILLIAMS PETER (US); KRONE JENNIFER REEVE (US)) 28 November 1996 (1996-11-28) claims 1-3; examples 1-8	1-9

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 00/00268

Patent document cited in search report	Publication dat	Patent family member(s)	Publication date
WO 9856028 A	10-12-1998	AU 7713198 A	21-12-1998
		EP 0990255 A	05-04-2000
WO 9701755 A	16-01-1997	EP 0835446 A	15-04-1998
		JP 11509314 T	17-08-1999
WO 9743301 A	20-11-1997	AU 2889097 A	05-12-1997
WO 9637777 A	28-11-1996	NONE	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P49375PC00		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL00/00268	26/04/2000	26/04/1999
International Patent Classification (IPC) or national classification and IPC G01N33/566		
Applicant SCREENTEC B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/11/2000	13.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Thumb, W Telephone No. +49 89 2399 7350

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL00/00268

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-16 as originally filed

Claims, No.:

1-12 as originally filed

Drawings, sheets:

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 11, 12.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 11, 12.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statem nt

1. Statement

   Novelty (N) Yes: Claims 1-10

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-10
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
s e separate sheet

R It m V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: BLOM, KARL F.; LARSEN, BARBARA S.; MCEWEN, CHARLES N.: 'Determining Affinity-Selected Ligands and Estimating Binding Affinities by Online Size Exclusion Chromatography/Liquid Chromatography-Mass Spectrometry' JOURNAL OF COMBINATORIAL CHEMISTRY, vol. 1, no. 1, January 1999 (1999-01), pages 82-90, cited in the application

D2: NEDVED, MICHAEL L.; HABIBI-GOUDARZI, SOHRAB; GANEM, BRUCE; HENION,: 'Characterization of Benzodiazepine 'Combinatorial' Chemical Libraries by Online Immunoaffinity Extraction, Coupled Column HPLC-Ion Spray Mass Spectrometry-Tandem Mass Spectrometry' ANALYTICAL CHEMISTRY, vol. 68, no. 23, 1 December 1996 (1996-12-01), pages 4226-4236, cited in the application

D3: HUYER, GREGORY; KELLY, JOHN; MOFFAT, JASON; ZAMBONI, ROBERT; JIA, ZONGCHAO; GRESSER, MICHAEL J.; RAMACHANDRAN, CHIDAMBARAM: 'Affinity selection from peptide libraries to determine substrate specificity of protein tyrosine phosphatases' ANALYTICAL BIOCHEMISTRY, vol. 258, no. 1, 1998, pages 19-30, cited in the application

D4: WO-A-97/01755

2. Novelty

Independent claim 1 and dependent claims 2-10 are novel (Article 33(2) PCT), since an on-line method comprising a fractionation step, addition of an affinity molecule to the analyte-containing effluent of the fractionation step, followed by two separation steps and subsequent mass spectroscopy of the analyte, is not disclosed in the prior art known to the Examining Authority.

3. Inventive step

3.1 Independent claim 1 is considered as being inventive within the meaning of Article 33(3) PCT for the following reasons:
Claim 1 refers to an on-line method for detecting an analyte, said analyte binding to an affinity molecule (see item 2. above).
On-line methods comprising affinity-binding, separation steps and detection via mass spectroscopy are well known in the art.
For example, document D1, which is considered to represent the most relevant state of the art, describes an on-line system which includes size exclusion chromatography (SEC) isolation of affinity-selected ligands, combined with reverse phase liquid chromatography-mass spectrometry (LC-MS). Binding of compounds to human metalloprotease (MMP3) is detected. Defined quantities of a mixture of compounds and the protein are combined, the resulting protein/ligand complexes and unbound ligands are separated by SEC (corresponding to the first separation step of claim 1), and the eluted complexes are collected on a protein trap. The trapped material is desalted and the complexes are denatured using a LC gradient (dissociation step of claim 1). The bound compounds are released into an LC-MS system, wherein the LC column is a reversed phase C8 column (corresponding to the second separation step of claim 1), and are subsequently detected by mass spectrometry (page 83, column 1, last paragraph - column 2, paragraph 3). The method has a low background, as shown in experiments with an active and inactive compound, including positive and negative controls (see page 89, column 1, line 39 - column 2, line 10; figures 9 and 10).
Document D2 describes an on-line system to detect analyte-binding to an antibody, comprising coupling of an affinity column, used to bind an antibody-analyte complex, a restricted access media (RAM) column, separation of the antibody from the analyte, a reversed phase C8 column and detection via mass spectrometry (page 4228, column 2, lines 18-28; page 4231, column 1, lines 7-48; figure 1).

3.2 The subject-matter of claim 1 differs from the teaching of D1 in that a step fractionating the analytes prior to the reaction with the affinity protein is included in the method and that the first separation step comprises the use of a restricted-access support.

In view of the arguments presented by the applicant the underlying problem may be seen in providing a sensitive method allowing for detection of ligands of an affinity molecules at low concentration/low affinity of said ligand.
Fractionating a solution containing different analytes prior to investigating binding of individual components to a target molecule is a standard technique in the art. A multitude of methods are well known to the person skilled in the art, including size exclusion chromatography, ion exchange chromatography and HPLC.
Restricted access media (RAM) stationary phases are also known in the art. For example, document D2 describes the use of a RAM support to separate an analyte from an antibody specific thereto (page 4228, column 2, lines 21-25; page 4229, column 2, last paragraph).
Document D4 describes the theoretical basis of restricted access media-based chromatography (page 22).
However, both D2 and D4 refer to separation of receptors from their ligands.
As also argued by the applicant, it is not mentioned in the prior art that RAM columns could be used to efficiently separate ligand-affinity molecule complexes from contaminating molecules in an on-line detection method, whereby the short separation times necessary decrease the dissociation of the complex between the ligand and the receptor (a problem mentioned in D1, page 82, column 2, lines 6-28), especially in cases where the ligand to be identified using the subsequent mass spectroscopy step has a low affinity for the receptor.
Therefore, no direct indication is given in the state of the art that would prompt a skilled person to use the sequence of separation and detection steps as defined in claim 1 in order to arrive at a solution to the above-stated problem, i.e. a sensitive method of identifying ligands binding to affinity molecules.
Claim 1 is therefore considered as being inventive in the sense of Article 33(3) PCT.

3.3 Dependent claims 2-10 refer to further specific embodiments of the method of claim 1 and consequently also meet the requirements of Article 33(3) PCT.

R It m VIII

Certain observations on the international application

1. The wording "affinity molecule to said effluent" is not clear (Article 6 PCT), since said effluent or components included therein are either not defined or only defined later in the claim ("analytes in the effluent").

2. Claim 5 is not clear within the meaning of Article 6 PCT, since "a combinatorial chemistry system" as such is not considered to be a fractionating step.

3. Abbreviations used in the claims (e.g. claim 6) should be written in full to avoid any unclarity.